Characterization and expression of the bovine growth hormone-releasing hormone (GHRH) receptor

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Abstract

The hypothalamic hormone, growth hormone-releasing hormone (GHRH) and its pituitary receptor are principal regulators of pituitary growth hormone (GH) synthesis and release. In the present study, we cloned and sequenced a complete bovine pituitary GHRH receptor cDNA in order to study its expression in cattle. The lengths of the exons in the bovine GHRH receptor gene were determined by comparison of the cloned cDNA with genomic sequences obtained from a bovine genomic library clone. As in other species, the bovine cDNA sequence encodes a 423-amino acid protein containing seven hydrophobic domains characteristic of a G protein-coupled receptor. The predicted bovine amino acid sequence shares 93, 90, 89, 87, and 85% identity with the ovine, porcine, human, rat and mouse sequences, respectively. Expression of the receptor in bovine ileum, ovary, anterior pituitary, testis, hypothalamus, pancreas and liver was examined by RT-PCR. Of those tissues examined, GHRH receptor expression was detected in the anterior pituitary gland and hypothalamus. To gain a better understanding of GHRH receptor gene regulation in ruminants, we examined the effect of bovine somatotropin (bST) treatment on pituitary GHRH receptor expression in dairy heifers using relative and real-time RT-PCR. In the present study, bST treatment of dairy heifers resulted in no significant decline in pituitary GHRH receptor expression.

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1. Introduction

The hypothalamic hormone, growth hormone-releasing hormone (GHRH), is the principal stimulator of pituitary growth hormone (GH) synthesis and secretion [1]. Its pituitary receptor is well characterized as a member of the superfamily of G protein-coupled receptors, containing seven transmembrane domains [2]. The GHRH receptor appears to be expressed predominantly in pituitary tissue [2–4], although there is evidence of synthesis of its ligand in peripheral tissues including ovary [5], pancreas, gastrointestinal tract [6], placenta and testes [7]. Low-level expression of GHRH receptor has been detected in placenta, ovary [8], testis [8,9], kidney, hypothalamus and the gastrointestinal tract [10]. Interestingly, splice variants of the GHRH receptor have been demonstrated by PCR in human extrapituitary cells including liver, pancreas and prostate, but were non-detectable by Northern blotting [11]. The functions of GHRH receptor gene product outside of the pituitary gland and hypothalamus have not been determined.

The human GHRH receptor gene promoter and gene structure, through exon 10 (>8 kb), was recently described [12] and has been found to consist of 13 exons [10]. Most recently, the 14 exons of the rat GHRH receptor gene (∼15 kb) and its promoter were characterized [13]. To date, the GHRH receptor cDNA has been sequenced in human [2,3], pig [14], rat [2,4], mouse [4], sheep and cattle [15]. Sequencing and expression of the bovine GHRH receptor gene has not been well characterized, although the gene was recently mapped to bovine chromosome 4 by linkage analysis [16].

Regulation of pituitary GHRH receptor gene expression by GH has been studied in rodents. For instance, suppression of pituitary GHRH receptor mRNA by GH autofeedback was demonstrated in transgenic mice expressing human GH targeted to the hypothalamus [17] and in rats treated with recombinant human GH [18,19]. To date, no studies have examined the effects of GH on pituitary GHRH receptor expression in ruminants. The purpose of the present study was to clone and sequence the bovine pituitary GHRH receptor cDNA and to examine the effects of short- and long-term bovine somatotropin (bST) treatment on its expression.

2. Materials and methods

2.1. Tissue collection

To clone the bovine pituitary GHRH receptor cDNA and to examine tissue distribution of GHRH receptor expression, anterior pituitary gland, hypothalamus, testis and liver were collected from Holstein bull calves (<3 days old) and ileum, ovary, and pancreas were collected from post-pubertal Holstein heifers at slaughter. All tissues were immediately frozen in liquid nitrogen and stored at −80°C until extraction of total RNA was performed.

2.2. Cloning and sequencing of bovine pituitary GHRH receptor cDNA

Total RNA was isolated from bovine anterior pituitary and first strand cDNA was synthesized with MMLV reverse transcriptase using an oligo d(T) primer (RT-PCR Kit, Stratagene,

La Jolla, CA), according to manufacturer's instructions. An 1133 bp fragment was amplified by PCR with specific primers designed from conserved sequences of porcine (GenBank accession nos. U49435 and L11869), human (L09237 and L01406) and ovine (AY008834) GHRH receptor cDNAs. Primer sequences were 5′-ATGGGCAGCAGGGTGTGGGGCG-3′ (sense) and 5′-AAGCAGTACAGGATAGCAAC-3′ (antisense). PCR-amplification was performed using the MJ Research DNA Engine and the following cycling conditions: 94°C for 1 min, 53°C for 1 min, and 72°C for 2 min for 30 cycles, followed by 10 min at 72°C in a 100 μL reaction volume (RT-PCR Kit). The 1133 bp fragment was cloned into a plasmid vector, sequenced and used as a hybridization probe to screen a bovine pituitary lambda cDNA library (Stratagene) and a bovine lambda genomic library (Stratagene).

Four positive lambda clones were purified from the cDNA library and the inserts were sequenced in both directions using T3, M13(−20), and gene-specific primers. A single lambda clone was isolated from the genomic library and directly sequenced by primer walking. Exon sizes were determined by loss of identity between the genomic and cDNA nucleotide sequences.

Rapid amplification of cDNA ends (RACE) was performed to obtain the 5′ region of the cDNA through the putative initiation codon using the SMART^{TM} RACE cDNA Amplification Kit (Clontech, Palo Alto, CA). The gene-specific primer sequence used for 5′ RACE was 5′-GACATCACATTCTGGGTGCACGTGG-3′.

An automated DNA sequencer (ABI-PRISM 377, PE Biosystems, Foster City, CA) was used for all sequencing. The reported sequence for bovine pituitary GHRH receptor is based on the sequence obtained from 5′ RACE, RT-PCR of the GHRH receptor from pituitary total RNA, and bovine pituitary cDNA library clones. Repeated PCR-amplification of the region and direct sequencing of the PCR products in both directions confirmed accuracy of the sequence obtained by RT-PCR. Sequence comparisons were conducted using Biology WorkBench 3.0-BLASTP (http://biology.ncsa.uiuc.edu).

2.3. Examining tissue distribution of GHRH receptor expression

First strand cDNA synthesized from bovine ileum, ovary, anterior pituitary, testis, hypothalamus, pancreas and liver (described above) was used as template for PCR. Primer pairs used for PCR were 5′-GTACTGCTTCCTCAACCAAG-3′ (sense) and 5′-CAAGAGG-ATCCCTGCACAGAC-3′ (antisense). Cycling conditions used to amplify a 524 bp fragment of GHRH receptor cDNA were as follows: 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min for 30 cycles, followed by 10 min at 72°C in a 100 μL reaction volume (RT-PCR Kit). PCR products (10 μL) were separated by agarose gel electrophoresis. The remaining 90 μL PCR reaction volume from the pituitary sample was ethanol precipitated and directly sequenced to confirm PCR-amplification of bovine GHRH receptor. As a positive control of reverse transcription preparations, the GAPDH constituitively expressed gene was PCR-amplified from first strand cDNA synthesized from each tissue. Primer sequences [5′-GGCATCGTGGAGGGACTTATG-3′ (sense) and 5′-GCCTGCTTCACCACCTTCTTG-3′ (antisense)] were designed from the bovine GAPDH cDNA sequence (GenBank accession no. U85042) to amplify a 290 bp product. PCR-amplification was performed using the MJ Research DNA Engine according to the manufacturer's protocol using the following cycling conditions: 94°C for 1 min, 61°C

for 1 min, and 72°C for 2 min for 30 cycles, followed by 10 min at 72°C in a 100 μL re-action volume (RT-PCR Kit). PCR products (10 μL) were separated by agarose gel elec-trophoresis. The remaining 90 μL PCR reaction volume from the pituitary sample was ethanol precipitated and directly sequenced to confirm PCR-amplification of bovine GAPDH.

2.4. Determining the effects of GH treatment on bovine GHRH receptor expression

As part of a larger study, 12 Holstein heifers (approximately 3 months of age) were un-treated ($n = 6$) or treated with bST (250 mg Posilac every 14th day; Monsanto Company, St. Louis, MO) for 2 months ($n = 3$) or 7 months ($n = 3$). The concentrations of serum GH and plasma IGF-I of representative animals from the larger study ($n = 5$ control; $n = 4$ bST) were determined by radioimmunoassay as previously described [20,21] to confirm that bST treatment resulted in elevated circulating concentrations of GH and could potentially affect pituitary GHRH receptor mRNA expression. The pituitary gland was collected from all 12 animals at slaughter, weighed, immediately frozen in liquid nitrogen, and then stored at −80°C until extraction of total RNA was performed. Relative RT-PCR using Quantum RNA[TM] 18S Internal Standards (Ambion, Austin, TX) was performed in duplicate accord-ing to manufacturer's instructions to characterize the expression profile of bovine pituitary GHRH receptor in control versus bST-treated animals after 2 and 7 months of bST treatment. The Quantum RNA kit employs a competimer for 18S ribosomal RNA, which is a primer pair with 3′ end modification that prevents extension by DNA polymerase. This approach permits specific reduction of 18S amplification efficiency to the same linear range as target gene amplification and maintains relative quantitation. For each reverse transcription reaction, 2 μg of total RNA was used. PCR products were quantified in an ethidium bromide stained agarose gel using a ChemiImager 4000 (Alpha Innotech Corporation, San Leandro, CA) with Alpha Ease 4.0 image analysis software. The linear range of the assay was 30–34 cycles of PCR-amplification (Fig. 1). For the quantitation experiment, 32 cycles of PCR-amplification and a 2:8 18S primer:competimer ratio were used to obtain similar yields of GHRH receptor and 18S ribosomal RNA. Pituitary GHRH receptor expression was calculated for each sam-ple as a ratio of the GHRH receptor fragment intensity to the 18S ribosomal RNA fragment intensity.

To further verify the effect of bST treatment on GHRH receptor expression, real-time one-step RT-PCR was performed using the QuantiTect SYBR Green RT-PCR kit (Qiagen, Valencia, CA) according to manufacturer's instructions and the DNA Engine Opticon Con-tinuous Fluorescence Detection System (MJ Research Inc., Waltham, MA). Reactions were performed in a 25 μL volume using 250 ng of total RNA. Cycling conditions were as follows: 50°C for 30 min, 95°C for 15 min, followed by 45 cycles of 94°C for 15 s, 58°C for 30 s, and 72°C for 30 s. Standard curves were generated in triplicate for both GHRH receptor and GAPDH using varying quantities of total pituitary RNA. Samples were analyzed in duplicate and the coefficient of variation between replicates for cycle threshold was less than 3.1%. Pituitary GHRH receptor expression was calculated for each sample as a ratio of the quantity of GHRH receptor mRNA to GAPDH mRNA.
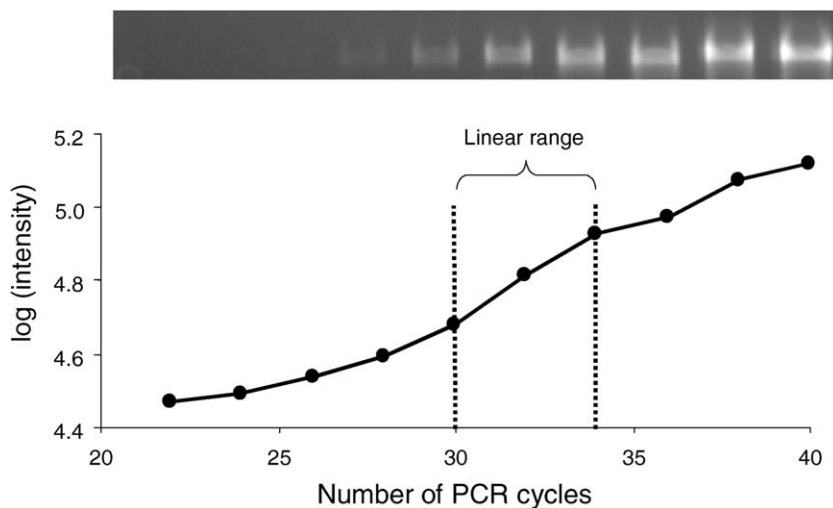
Fig. 1. Linear range of relative RT-PCR analysis by ethidium bromide. A representative RNA was used as template for the reverse transcription reaction. A master PCR mix was divided into 10 aliquots and subjected to PCR. Aliquots were removed from the thermocycler at the indicated cycles and resolved by electrophoresis in a 2% agarose gel stained with ethidium bromide. The products were then quantified with image analysis software.

2.5. Statistical analysis

Statistical analyses were conducted using the SAS System Version 8.2 (SAS Institute Inc., Cary, NC). The effects of bST and time on GHRH receptor expression were evaluated by two-way ANOVA using the GLM procedure. An alpha level of 0.05 was used for all statistical inferences.

3. Results

The bovine pituitary GHRH receptor cDNA sequence was obtained from three sources: (1) the 5′-RACE product (nucleotides 1–87); (2) the 1133 bp hybridization probe (nucleotides 45–1135); and (3) the four positive clones isolated from a pituitary cDNA library (nucleotides 482–1723). The putative open reading frame is 1269 bp. Alignment of our cDNA sequence with bovine cDNA sequences reported in GenBank (accession nos. AY008835 and AB022597) shows over 99% identity. The predicted amino acid sequence of bovine GHRH receptor is 93, 90, 89, 87 and 85% identical to the ovine, porcine, human, rat and mouse sequences, respectively (Fig. 2), providing additional evidence of the highly conserved structure and function of the receptor protein across species.

The lengths of putative exons 2–12 were determined by loss of identity between the lambda genomic clone and cDNA sequences and the size of putative introns 1–12 were estimated by PCR or sequencing (Table 1). Intron/exon borders beyond putative exon 12 could not be determined due to a lack of genomic sequence required for comparison to the cDNA sequence.

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Bovine  MDSRVWGACVLCLLGPLPIVLGHVHPECDVITQLREDEQACLQAAEGMPNSTLGCPRIWD      60
Ovine   -G----------------------------------------------------------
Porcine ---G—A---IF---SS—VA----------F--------RT-----DR-A--SS----T--
Human   --R-M---H-F-V-S---T----M-----F--------S------E---T-----AT--
Rat     --GLM-ATRI----SLCGVT---L-L---F-----D--L-------TN-TS----GT--
Mouse   ---LL-ATW-----NLWGVA---L-L---F-----D--L--------TN--SM---GT--

Bovine  GLLCWPTAGSGEWVSLPCPAFFSHFSSEPGAVKRDCTIAGWSEPFPPYPEACPVPLELLT     120
Ovine   ------M------------------L---------------------------------
Porcine ---------P----T---------------L-----TT--------------------
Human   --------------T----D--------S---------T----------V---------A
Rat     ------PT---Q-------E-----G-DT-F-------T---N------V----------
Mouse   ------PT---Q-------E-----G-D----------T---D------V----------

Bovine  EEKSYFSAVRIIYTMGHSVSAAALLVAIIILVALRRLHCPRNYIHTQLFITFILKAAAVF     180
Ovine   ----------V----------------------------------T----------
Porcine D------T---V--T------V--F----A----------------S---A------G---
Human   --E----T-K----V---I-IV--F---T-------------V-----T------G---
Rat     K------T-K----T---I-IV--C---A------------------A------S---
Mouse   -------T-K----T---I-IV--C---A------------------A------S---

Bovine  LKDATLFHQENTDHCSFSTVLCKVSVATSHFATMTNFSWLLAEAVYLTCLLVSTLPSTRR     240
Ovine   --------R--M----------A--TA---------------------A--------
Porcine ----A---S---------------------------------------A--S-----
Human   ----A---SDD---------------A------------------N---A—S-----
Rat     ----AI-QGDS-----M----------I--L----------------S---A--S-RSKP
Mouse   ----AV-QGDS-----M--I-------V----------------S---A--S-RSKP

Bovine  VFWWLVLAAWGLPLLFTGMWVGCKLAFEDVACWDLDDSSPYWWIIKGPIVLSVGVNFGLF     300
Ovine   ----------------S-----------------------------------
Porcine A-------G---------T---------------------------------
Human   A-------G----V----T--S-------I-------T----------------
Rat     A-------G----V-C--T-----HS---TE-----N---C----------------
Mouse   A-------G----V-C--T----------T----------------------

Bovine  LNIIRILLRKLEPTQGSLHTQHQYWRLSKSTLLLIPLFGIHYVIFNFLPDSAGLDIRLPL     360
Ovine   --------------------P------------------------------
Porcine ------------A-------P----------------------------G-----
Human   -------V-----A-------S----------F---------I-------N---G-----
Rat     ----C--------A--G---RA-------------------I-------------V--
Mouse   ----C------G-A--G---RA-------------------I----------G-----

Bovine  ELGLGSFQGFIVAILYCFLNQEVRTEISRRWHGHDLELLPARVTHIKWTTPSHSRVKVLTSAC     423
Ovine   ---------------------------------P------R----
Porcine --------------------------------P-----WR--A--AK--R--A----TV-
Human   --------------------------K-----P-----WR-RA------R-AA-----M-
Rat     -------------V---------------K-Y---P------R-CTE----PR--L-----E-
Mouse   ----------V--V--------------K-Y---P------R-CTE----PR-------E-
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Fig. 2. Predicted amino acid sequence alignment of bovine, ovine, porcine, human, rat and mouse pituitary GHRH receptor. Dashes (–) indicate identity with bovine sequence.

Comparison of exon length between the bovine and human genes [12] through exon 10 shows exon sizes are identical between those species.

Sequencing of the 290 and 524 bp products amplified from pituitary first strand cDNA confirmed amplification of bovine GAPDH and GHRH receptor, respectively. Expression of GHRH receptor mRNA in various bovine tissues, as determined by RT-PCR, is shown in Fig. 3. Evidence of GHRH receptor expression, as indicated by the presence of a 524 bp product, was confined to anterior pituitary and hypothalamic tissues (Fig. 3). As a positive control of RNA

Table 1
Length of putative exons and introns in the bovine GHRH receptor gene

| Exon				Intron		
No.	Location	Length (bp)	5′ Splice donor	No.	Length (bp)[a]	3′ Acceptor
I	1 . . . 79	?	CCG ATC	I	~3700	GTC CTG
II	80 . . . 182	103	ACC TTG G	II	127[b]	GC TGC CCT
III	183 . . . 290	108	GAG CCA G	III	~700	GG GCT GTG
IV	291 . . . 388	98	GAG GAG	IV	~1500	AAA TCC
V	389 . . . 486	98	GCT CTC AG	V	~650	G AGG CTC
VI	487 . . . 619	133	TCC ACT	VI	~1600	GTC CTG
VII	620 . . . 773	154	GCC TGG G	VII	~400	GG CTT CCT
VIII	774 . . . 834	61	GGT TGC	VIII	~680	AAG TTG
IX	835 . . . 904	70	GTT GGG	IX	~900	GTG AAC
X	905 . . . 996	92	CAG TAC TG	X	~460	G CGT CTC
XI	997 . . . 1126	130	TTC CAG	XI	~1000	GGC TTC
XII	1127 . . . 1168 . . .	42	CAA GAG	XII	~1900	GTG AGG
XIII	1169 . . .	?	–	–	–	–

?: Length unknown.

[a] Length of introns determined by PCR.

[b] Length of introns determined by sequencing.
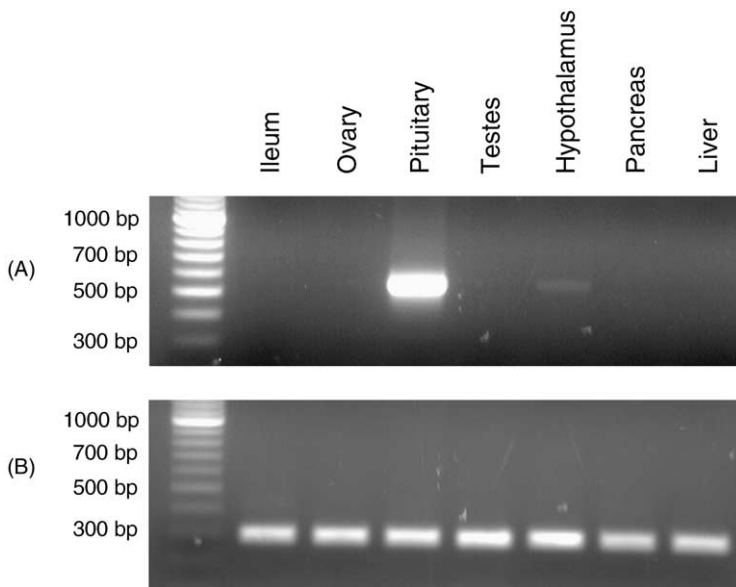
Fig. 3. Tissue-specific expression of bovine GHRH receptor and GAPDH mRNAs as determined by RT-PCR. Each RT-PCR product and 100 bp DNA ladder were electrophoresed in a 2% agarose gel stained with ethidium bromide. Panel A, the presence of a 524 bp product indicates expression of GHRH receptor message by that tissue. Panel B, the presence of a 290 bp product indicates expression of GAPDH message by that tissue.

Table 2
Mean (±SE) circulating concentrations of growth hormone (GH) and insulin-like growth factor-I (IGF-I) in representative control ($n = 5$) and bST-treated ($n = 4$) heifers after 2 and 7 months of treatment[a]

Variable	Treatment group		
	Control	bST	*P*-value
Serum GH (ng/mL), 2 months	5.2 ± 1.4	27.9 ± 11.1	0.14
Serum GH (ng/mL), 7 months	1.8 ± 0.2	28.4 ± 5.2	0.01
Plasma IGF-I (ng/mL), 2 months	88.5 ± 6.7	85.0 ± 8.6	0.75
Plasma IGF-I (ng/mL), 7 months	210.5 ± 28.3	197.7 ± 25.0	0.75

[a] Heifers received 250 mg Posilac every 14th day.

isolation and cDNA synthesis from each tissue, GAPDH was amplified from each tissue using bovine GAPDH-specific primers. Products of the expected size for GAPDH (290 bp) were amplified from all tissues tested, indicating that failure to amplify a 524 bp product was due to absence of GHRH receptor transcript in that tissue, and not problems with RNA isolation or first strand cDNA synthesis.

Mean (±SE) serum GH concentrations and plasma IGF-I concentrations of representative control ($n = 5$) and bST-treated heifers ($n = 4$) are shown in Table 2. Treatment with 250 mg bST every 14th day produced no difference ($P = 0.14$) in mean circulating concentrations of GH compared to control heifers by 2 months, but resulted in an 15-fold increase ($P = 0.01$) in serum GH concentration after 7 months of treatment. Mean plasma IGF-I concentrations of bST-treated heifers were similar ($P = 0.75$) to control heifers after 2 and 7 months of treatment. Mean pituitary weights in control versus bST-treated heifers after 2 and 7 months of bST treatment were not significantly different (data not shown).

Results of relative RT-PCR comparing GHRH receptor expression in control versus bST-treated heifers after 2 and 7 months of treatment are shown in Fig. 4. There was no bST × time interaction ($P = 0.55$) on GHRH receptor expression. Treatment with 250 mg bST every 14th day resulted in no significant change in pituitary GHRH receptor expression compared to control heifers after 2 or 7 months of treatment ($P = 0.20$). In control heifers, mean GHRH receptor expression tended to decline over time ($P = 0.09$). Results of real-time RT-PCR were consistent with the findings using relative RT-PCR, showing no bST × time interaction ($P = 0.51$) and no effect of bST ($P = 0.89$) or time ($P = 0.39$) on GHRH receptor expression.

4. Discussion

In the present study, we describe the sequence of the bovine GHRH receptor cDNA and the intron/exon structure of the GHRH receptor gene. Our results indicate that the bovine receptor sequence is quite similar to sequences previously described for human [2,3], pig [14], mouse [4] and rat [2,4] and compared to other species, shares the greatest degree of identity with ovine GHRH receptor [15]. As in humans, pigs and rodents, the bovine GHRH
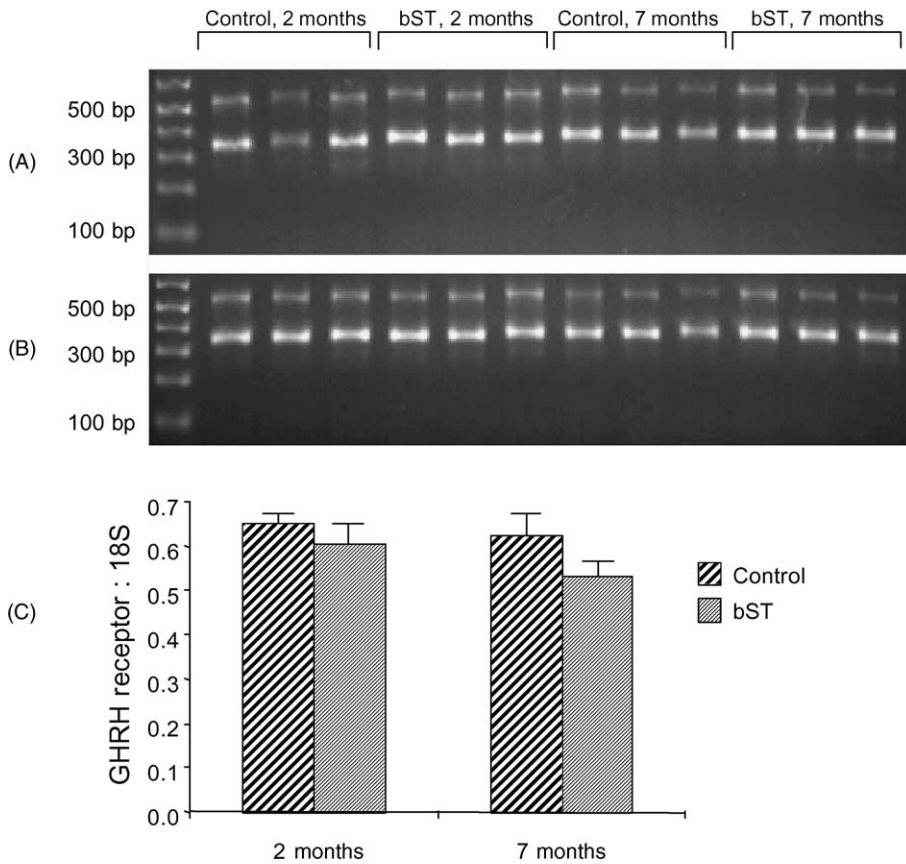
Fig. 4. Expression of bovine GHRH receptor message in control vs. bST-treated heifers after 2 and 7 months of treatment (250 mg Posilac every 14th day) as determined by relative RT-PCR. Each RT-PCR product was separated by electrophoresis in a 2% agarose gel stained with ethidium bromide. The 524 bp product indicates expression of GHRH receptor message and the 324 bp product indicates expression of 18S ribosomal RNA message. Samples were analyzed in duplicate and are shown in Panels A and B. Signal intensity for each product was quantified using image analysis software. Panel C, mean ± SE expression levels of pituitary GHRH receptor for each treatment calculated as the ratio of the signal for GHRH receptor to the signal obtained for the 18S ribosomal RNA ($n = 3$ animals per treatment group).

receptor cDNA encodes a 423-amino acid protein, whereas in sheep and goats, the receptor is truncated at the carboxyl terminus to a 407-amino acid form [15]. Alignment of our sequence with another bovine sequence reported in GenBank (accession no. AY008835) identifies three amino acid differences (residues 57, 192, and 322). It is not known whether these variations represent true polymorphisms in the bovine population; however, these amino acid changes do not affect the hydropathy profile of the protein as determined by the Biology WorkBench 3.0-GREASE: Kyte-Doolittle Hydropathy Profile ([22]; http://biology.ncsa.uiuc.edu).

Expression of GHRH receptor is predominantly in the pituitary gland [2–4] and is easily detected by Northern analysis. However, low-level expression in extrapituitary tissues

such as hypothalamus [23], renal medulla, renal cortex, gastrointestinal tract [24], placenta [8] and gonads [8,9] has been detected by the more sensitive techniques of RT-PCR and RNAase protection assay. Our detection of GHRH receptor expression in pituitary and hypothalamic tissues is consistent with previous studies, although GHRH receptor was not detected in bovine ileum, ovary or calf testis by RT-PCR in the present study. Low-level expression of GHRH receptor was detected in rat ovary by RNAase protection assay [8], but not in bovine ovarian tissue by RT-PCR [25]. Thus, it is possible that expression of GHRH receptor in gonad is unique to rats or that expression in bovine testis is age-dependent.

To our knowledge, this is the first study to examine the effects of GH treatment on pituitary GHRH receptor mRNA expression in cattle. However, suppression of pituitary GHRH receptor mRNA by GH treatment has been demonstrated in rats and mice where the effects appear to be mediated by short-loop feedback of GH on GHRH release [17,18,26]. For instance, Horikawa *et al.* [18] demonstrated suppression of GHRH receptor mRNA in rats treated with GH alone and in GHRH-immunized rats, indicating that reduction of GHRH, either by negative feedback effects of GH or immunoneutralization, results in decreased GHRH receptor expression. Further, transgenic mice expressing human GH in the hypothalamus exhibit a dwarf phenotype, GH and IGF-I deficiency, and significantly reduced hypothalamic GHRH mRNA and reduced pituitary GHRH receptor mRNA [17], suggesting negative feedback effects of GH on GHRH release and subsequent suppression of GHRH receptor expression. Studies of the spontaneous dwarf rat, which fails to produce GH due to a premature stop codon in the GH transcript, show that GH treatment suppresses GHRH and GHRH receptor mRNA and stimulates expression of neuropeptide Y (NPY) and somatostatin mRNA [26]. Kamegai *et al.* [26] propose that in rats, elevated GH activates hypothalamic NPY neurons, which then stimulate somatostatin neurons. Somatostatin then inhibits release of GHRH, resulting in a reduction in pituitary GHRH receptor expression.

Thus, based on studies in rodents, it was expected that bST treatment of dairy cattle would suppress pituitary GHRH receptor mRNA *via* a similar mechanism. However, in the present study, although bST treatment of dairy heifers for 7 months increased circulating concentrations of GH 15-fold compared to controls, no significant reduction in GHRH receptor expression was detected in bST-treated heifers. Pituitary weights of bST-treated heifers were not significantly different from controls suggesting that bST treatment at this dosage did not result in negative feedback on GHRH release. Unfortunately, hypothalamic expression of GHRH mRNA was not examined in the present study. Treatment with bST resulted in no significant differences in IGF-I concentration, which although surprising, is consistent with observations of bST-treated bull calves [27].

Our results suggest that GH treatment may not suppress GHRH receptor expression in cattle as previously described in rodents. Likewise, no relationship was observed between pituitary GHRH receptor mRNA and GH mRNA or circulating concentrations of GH in sheep [28]. Therefore, further research is needed to determine the role of hormones of the hypothalamo–pituitary–GH axis in pituitary GHRH receptor regulation in ruminants. Sequencing of the bovine GHRH receptor cDNA, characterization of the gene, and the RT-PCR assays described in the present investigation will assist in the study of GHRH receptor gene regulation in cattle.

Acknowledgments

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